compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent; and

cleaving at least one of the cleavable peptidyl fragments.--

REMARKS

Claims 78-130 are pending. Claims 98, 100, 101, 105, 110, 115, 117-119 and 130 are herein canceled. Applicant presents amendments to base claims 78 and 123 and dependent claims 79-81 and 91. Claim 131 is newly presented.

Upon entry of these amendments, claims 78-97, 99, 102-104, 106-109, 111-114, 116, 120-129, and 131 would be pending.

Claims 78-130 were subject to the following Restriction Requirement pursuant to 37 CFR §1.499 as set forth by the Examiner:

	CLAIMS	Subject Matter	SEQ ID NO:	SEQ ID NO
I	1-102, 104-108, 111-113, 116-127, and 129 (each in part)	drawn to a method of preparing a bioactive protein comprising expressing a recombinant protein comprising a first peptidyl fragment and second peptidyl fragment wherein the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment	1	4
II	1-102, 104-108, 111-112, 114, 116-127, and 129 (each in part	" "	2	4
III	1-102, 104-107, 110-112, 115-127, and 129 (in part)	" "	3	4
IV	1-101, 103-108, 111-113, 116-126, and 128-129 (each in part)	" "	1	5
V	1-101, 103-107, 109, 111-112, 114, 116-126, and 128-129 (each in part)	" "	2	5
VI	1-101, 103-107, 110-112, 115-126, and 128-129 (each in part)	" "	3	5
VII	130	Assay	—	—

Applicant has elected Group IV with traverse and a request for reconsideration as discussed below.

The Amendments to the Claims

The preamble of claim 78 was amended to recite "a protein having a correctly folded human insulin precursor." Support for this subject matter is found in the specification, inter alia, at p. 24, lines 22-30.

Claims 78 and 123 were amended to recite "from N-terminus to C-terminus." Support for this subject matter is found in the specification at p. 10, lines 5-6.

Claims 78 was amended to recite "a first peptidyl fragment which has an amino acid sequence which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment." Support for this subject matter is found in the specification, *inter alia,* at p. 10, lines 5-19.

Claim 78 was also amended to recite "whereby the protein is correctly folded." Support for this subject matter is found as above.

Claim 123 was amended to recite "a first peptidyl fragment which comprises an amino acid sequence at least 60% identical to the first 20 N-terminal amino acids of SEQ ID NO: 1." Support for this subject matter is found in the specification, *inter alia,* at p. 10, lines 5-19.

Claims 78 and 123 were also amended to recite a "human insulin precursor." Support for this subject matter is found, *inter alia,* in the specification at p. 17, first two lines and in claims 101 and 1126, respectively.

Claim 78 was also amended to recite "wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of the recombinant protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same recombinant protein lacking the first peptidyl fragment with the chaotropic agent; and contacting the recombinant protein with an aqueous medium comprising the chaotropic agent." Support for this subject matter is found, inter alia, in the specification at p. 10, lines 19-34.

Claim 79 was amended to recite "wherein the aqueous medium comprises at least one chaotropic agent." This amendment finds support in the previous version of claim 79.

Claim 80 was amended to recite "wherein one of the at least one chaotropic auxiliary agent is urea." Support for this subject matter is found, *inter alia*, in previous version of claim 80.

Claim 81 was amended to change its dependency from claim 79 to claim 80. Claim 80 provides the antecedent basis for "urea". Support for this subject matter is found in the previous version of claim 81.

Claim 91 was amended to recite "contacting" as the antecedent reference rather than "causing." Support for this subject matter is found in the previous version of claim 91.

Claim 131 is a new independent claim. Claim 131 recites the elements of amended claim 78 and then further recites "and cleaving at least one of the cleavable peptidyl fragments." Support for this subject matter is found, *inter alia,* as set forth above for claim 78 and claims 120 and 121.

Applicant believes the amendments to the claims add no new matter and respectfully request their entry.

TRAVERSAL OF THE RESTRICTION REQUIREMENT PURSUANT TO 37 CFR 1.499

A. Restriction between a first peptidyl fragment of SEQ ID NO:1 and a first peptidyl fragment of SEQ ID NO:2 (Groups IV and V).

SEQ ID NO: 1 is identical to the first 49 amino acids of SEQ ID NO:2. Restriction between these sequences is therefore improper. A first peptidyl fragment having an amino acid sequence of SEQ ID NO:1 would encompass a first peptidyl fragment having an amino acid sequence of SEQ ID NO:2. Their subject matter is related as genus and species. Thus, it is incorrect to say that the same technical features are not required of Groups I and II or Groups IV and V.

In view of the above, Applicant respectfully requests that the above restriction be reconsidered and withdrawn and that Groups IV and V be rejoined for examination.

B. Restriction between a second peptidyl fragment of SEQ ID NO:4 and a second peptidyl fragment of SEQ ID NO:5.

The amino acid sequence of SEQ ID NO: 5 is identical to the amino acid sequence of SEQ ID NO:4 through positions 1-31 and positions 66-86. These sequences are very closely related as product and precursor. The intervening amino acid sequence from positions 32 through 65 of SEQ ID NO:4 is normally removed during the processing of human insulin by the cleavage of the dibasic amino acid residues about positions 31 and 65. The peptide of SEQ ID NO:5 is then formed by the splicing together of the two cut ends of the precursor. The peptidyl fragments of SEQ ID NO:4 and SEQ ID NO:5 involve the same cysteinyl bridges and same bioactive conformations whose formation is mediated by the first peptidyl fragment. Thus, it is incorrect to say that the same technical features are not required of Groups I and IV. In view of the above, Applicant respectfully requests that the above restriction requirement be reconsidered and that Groups I and IV be rejoined.

In view of all of the above, Applicant therefore respectfully requests that the subject matter of Groups I and II and IV and V be rejoined and examined in this application.

C. Second peptidyl fragment which is a Human Insulin Precursor.

Claims 101 and 126 were drawn in part to "human insulin precursors." Peptidyl fragments of SEQ ID NO:4 and SEQ ID NO:5 are species of such human insulin precursors. The restriction requirement proposed by the Examiner did not address the "insulin precursor" subject matter. MPEP §1850 sets forth:

> If the independent claims avoid the prior art and satisfy the requirement of unity of invention, no problem of lack of unity arises in respect of any claims that depend on the independent claims. In particular, it does not matter if a dependent claim itself contains a further invention. Equally, no problem arises in the case of a genus/species situation where the genus claim avoids the prior art.

The peptidyl fragments of SEQ ID NO:4 and SEQ ID NO:5 are species of human insulin precursors and not appropriately restricted from "human insulin precursor" subject matter.

Thus, Applicant further requests reconsideration of the restriction requirement as the restriction requirement did not address the "human insulin precursor" subject matter in its full scope. This subject matter shares the same technical feature as that of SEQ ID NO:4 and SEQ ID NO:5. The human insulin precursor is defined (see p. 24, lines 13-30) as having the same cysteine bridges of SEQ ID NO:4 and SEQ ID NO:5 which are subject to intramolecular chaperoning by the first peptidyl fragment.

Applicant has amended base claims 78 and 123 to recite "a human insulin precursor." In light of the above, Applicant respectfully requests entry of this amendment.

D. The subject matter of new claim 131 involves the same special inventive feature.

New claim 131 recites an additional step over claim 78, namely the cleavage of the protein precursor at the cleavable peptidyl fragment recited in claim 78. Applicant submits that the subject matter of this claim involves the same inventive features as claim 78 and therefore respectfully requests this claim be entered for prosecution on the merits.

CONCLUSION

In view of the foregoing, Applicant believes all claims now pending in this Application are in condition for examination.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claims 98, 100, 101, 105, 110, 115, 117-119, and 130 have been canceled without prejudice.

Claims 78-81, 91 and 123 have been amended as follows:

78. (AMENDED) A method for preparing a [bioactive] protein having a correctly folded human insulin precursor [comprising at least one cysteine bridge] comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus, a first peptidyl fragment which has an amino acid sequence which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment, a second peptidyl fragment [comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment] which is a human insulin precursor, and at least one cleavable peptidyl fragment linking the first and second peptidyl fragments, wherein the first peptidyl fragment [mediates formation] is capable of increasing the yield of the bioactive conformation of the [second peptidyl fragment] insulin precursor formed upon contact of the recombinant protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same recombinant protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent; and

whereby the protein is correctly folded.

[causing the second peptidyl fragment to adopt the bioactive conformation.]

79. (AMENDED) A method according to claim 78, wherein [causing the second peptidyl fragment to adopt the bioactive conformation includes contacting the recombination protein with an] the aqueous medium [comprising] comprises at least one chaotropic auxiliary agent.

80. (AMENDED) A method according to claim 78, wherein one of the at least one chaotropic auxiliary agent is urea.

81. (AMENDED) A method according to claim [79] 80 wherein the urea is present in a concentration between about 2 M [to] and 8 M.

91. (AMENDED) A method according to claim 78, wherein [causing the second peptidyl fragment to adopt the bioactive conformation includes contacting] the recombinant protein is contacted with a mercaptan.

123. (AMENDED) A chimeric protein comprising from N-terminus to C-terminus:

a first peptidyl fragment which comprises an amino acid sequence at least 60% identical to the first 20 N-terminal amino acids of SEQ ID NO: 1;

a second peptidyl fragment comprising a human insulin precursor [an amino acid sequence] which exhibits insulin-like bioactivity when folded in a bioactive conformation; and

at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;

wherein the first peptidyl fragment [is selected such that it] mediates folding of the second peptidyl fragment to cause the second peptidyl fragment to adopt the bioactive conformation.

New claim 131 has been added:

131. (NEW) A method of making a correctly folded human polypeptide with insulin bioactivity, said method comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus, a first peptidyl fragment which has an amino acid sequence which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment, a second peptidyl fragment which is a human insulin precursor, and at least one cleavable peptidyl fragment linking the first and second peptidyl fragments, wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of the protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent; and

cleaving at least one of the cleavable peptidyl fragments.

APPENDIX I

PENDING CLAIMS AFTER ENTRY OF THIS AMENDMENT

78. (AMENDED) A method for preparing a protein having a correctly folded human insulin precursor comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus, a first peptidyl fragment which has an amino acid sequence which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment, a second peptidyl fragment which is a human insulin precursor, and at least one cleavable peptidyl fragment linking the first and second peptidyl fragments, wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of the recombinant protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same recombinant protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent;

and whereby the protein is correctly folded.

79. (AMENDED) A method according to claim 78, wherein the aqueous medium comprises at least one chaotropic auxiliary agent.

80. (AMENDED) A method according to claim 78, wherein one of the at least one chaotropic auxiliary agent is urea.

81. (AMENDED) A method according to claim 80 wherein the urea is present in a concentration between about 2 M and 8 M.

82. A method according to claim 81 wherein the urea is present in a concentration between about 3 M to 6 M.

83. A method according to claim 78 wherein the aqueous medium

further comprises a mercaptan.

84. A method according to claim 83 wherein the mercaptan is selected from the group consisting of dithiothreitol, dithioerythrol, 2-mercaptoethanol, cysteine, methyl thioglycolate, 3-mercapto-1,2-propanediol and 3-mercaptopropionic acid.

85. A method according to claim 83 wherein the mercaptan is 2-mercaptoethanol.

86. A method according to claim 79 wherein the aqueous medium has a pH between about 8 and 10.5.

87. A method according to claim 79 wherein the aqueous medium has a pH between about 9 and 10.

88. A method according to claim 79 wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.05 and 15 grams per liter.

89. A method according to claim 79 wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.5 and 5 grams per liter.

90. A method according to claim 79 wherein the recombinant protein is present in the medium in a concentration between about 2 and 3 grams per liter.

91. (AMENDED) A method according to claim 78, wherein the recombinant protein is contacted with a mercaptan.

92. A method according to claim 91 wherein the mercaptan yields less than 5 —SH radical of the mercaptan per cysteine residue of recombinant protein.

93. A method according to claim 91 wherein sufficient mercaptan is provided to yield between about 0.07 to about 1.0 —SH radical of the mercaptan per cysteine residue of recombinant protein.

94. A method according to claim 78, further comprising isolating a

portion of the expressed recombinant protein which is in the bioactive conformation.

95. A method according to claim 94 wherein isolating is performed by ultrafiltration.

96. A method according to claim 95 wherein ultrafiltration is performed at a pH between about 8 and 11.

97. A method according to claim 95 wherein ultrafiltration is performed at a pH between about 9 and 10.

99. A method according to claim 78 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

102. A method according to claim 78 wherein the second peptidyl fragment comprises SEQ. ID. No. 4.

103. A method according to claim 78 wherein the second peptidyl fragment comprises SEQ. ID. No. 5.

104. A method according to claim 78 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

106. A method according to claim 78 wherein the second peptidyl fragment comprises at least six cysteine residues which form three cysteine bridges.

107. A method according to claim 106 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

108. A method according to claim 107 wherein the first peptidyl fragment comprises SEQ. ID. No. 1.

109. A method according to claim 107 wherein the first peptidyl fragment comprises SEQ. ID. No. 2.

111. A method according to claim 107 wherein the first peptidyl

fragment is between 20 and 200 residues in length.

112. A method according to claim 78 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

113. A method according to claim 78 wherein the first peptidyl fragment comprises SEQ. ID. No. 1.

114. A method according to claim 78 wherein the first peptidyl fragment comprises SEQ. ID. No.2.

116. A method according to claim 78 wherein the first peptidyl fragment is between 20 and 200 residues in length.

120. A method according to claim 78 wherein the method further includes cleaving the at least one cleavable peptidyl fragment.

121. A method according to claim 78 wherein the at least one cleavable peptidyl fragment is an Arg or Lys residue.

122. A method according to claim 78 wherein the at least one cleavable peptidyl fragment is at least 2 amino acids in length where the C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

123. (AMENDED) A chimeric protein comprising from N-terminus to C-terminus:

a first peptidyl fragment which comprises an amino acid sequence at least 60% identical to the first 20 N-terminal amino acids of SEQ ID NO: 1;

a second peptidyl fragment comprising a human insulin precursor which exhibits insulin-like bioactivity when folded in a bioactive conformation; and

at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;

wherein the first peptidyl fragment mediates folding of the second peptidyl fragment to cause the second peptidyl fragment to adopt the bioactive conformation.

124. A protein according to claim 123 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

125. A protein according to claim 123 wherein the second peptidyl fragment is an insulin precursor.

126. A protein according to claim 123 wherein the second peptidyl fragment is an insulin precursor of human origin.

127. A protein according to claim 123 wherein the second peptidyl fragment comprises SEQ. ID. No. 4.

128. A protein according to claim 123 wherein the second peptidyl fragment comprises SEQ. ID. No. 5.

129. A protein according to claim 123 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

131. (NEW) A method of making a correctly folded human polypeptide with insulin bioactivity, said method comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus, a first peptidyl fragment which has an amino acid sequence which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment, a second peptidyl fragment which is a human insulin precursor, and at least one cleavable peptidyl fragment linking the first and second peptidyl fragments, wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of the protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent; and

cleaving at least one of the cleavable peptidyl fragments.